THE PERITONEAL EPITHELIUM OF SOME ITHACA AMPHIBIA.[1]

(*Necturus*, *Amblystoma*, *Desmognathus* and *Diemyctylus*.)

ISABELLA M. GREEN, A. B. (BUCHTEL), M. S. (CORNELL), AKRON, OHIO.

This investigation was undertaken with a view to determining the character of the epithelium of the peritoneum of the tailed *Amphibia* of the Cayuga lake basin. The *Amphibia* selected for this work represent the orders *Proteida* and *Urodela*. Of the former, only one species, *Necturus maculatus*, is included, while three families of the *Urodela* are represented, viz., *Amblystomidæ*, by *Amblystoma punctatum; Desmognathidæ*, by *Desmognathus fusca ;* and *Pleurodelidæ* by *Diemyctylus viridescens*. One of the *Anoura*, *Rana virescens*, was used by way of comparison.

It was the intention to make a study of the various species at all ages and at different seasons. This, however, has been impossible, except in a few cases. No *Necturi* could be examined after spawning and *Amblystoma punctatum* was the only species taken in the fall. Immature specimens of *Necturus* and *Desmognathus* and larvæ of *Desmognathus* were studied. By combining all the species we have a series composed of (1) larvæ ; (2) immature ; (3) mature before spawning ; (4) mature soon after spawning ; (5) mature taken in August ; and (6) mature taken in the fall.

As regards the use of the term epithelium, authors differ. Ranvier, Klein, Kolossow and Piersol apply it only to the covering of the mucous membranes, using endothelium for the covering of the serous membranes. Paladino, Neumann,

1. This paper was presented to Cornell University for the degree of M. S., in June, 1896.

Toureux, Hermann and Waldeyer discard the term endothelium, and call the covering of the serous as well as the mucous membranes epithelium. The term epithelium is used throughout this paper.

This investigation was carried on in the anatomical laboratory of Cornell University. I wish to express my appreciation of the facilities and material so generously placed at my disposal, and my gratitude to Dr. Wilder, Professor Gage and Instructor Hopkins, for their interest, encouragement and kindly criticism.

HISTORICAL.

For many years the serous membranes have been the subject of numerous investigations. Most of the work has been done upon mammals, but a number of articles have been written concerning the peritoneum of the *Amphibia.* The frog is the amphibian generally used, although Toureux ('74) worked upon the *Triton,* and Kolossow ('93) upon one salamander, two species of *Triton* and the *Axolotl,* in addition to the frog and toad.

Unfortunately their meagerness of data and details made it impossible to get the greatest good from the work of others. Often the statements were so vague that it was difficult to decide just what the author meant.

Among the first to work on the amphibian peritoneum was Mayer ('32 and '36). His results were not of great assistance, for although he spoke of finding cilia upon the peritoneum of the frog, the sex and age of the animal were not mentioned.

Valentine ('42 ?), in an article upon Ciliary Motion, enumerated the parts in a series of animals upon which cilia are found. Among them was mentioned the peritoneum of the tailed *Amphibians*, the *Triton* being given as an example. Cilia were found upon the serosa of the ovary.

Leydig ('57), in his Manual of Histology of Man and Animals, says that in the frog there are cilia upon some parts of the peritoneum, but not upon the mesentery, but does not

state the sex of the animal examined. He also found cilia upon the mesoarium.

The next work was of a more specific character, being a paper upon The Presence of Ciliated Epithelium upon the Peritoneum of the Female Frog, by Thiry ('62). He found cilia upon the peritoneum lining, the ventral wall of the body cavity, and upon the membranes around the mouth of the oviduct, but none upon the serosa of the liver, and does not mention their occurrence upon any other part of the peritoneum. Thiry proved by experiment that the current produced by the cilia was in the direction of the mouth of the oviduct.

V. Recklinghausen ('62) was the first to use silver nitrate upon the epithelium of the serous membranes as a means of bringing out the cell boundaries.

Ordmansson ('63) spoke of finding stomates in the peritoneum of the frog and rabbit.

In 1863, v. Recklinghausen demonstrated by a number of experiments the presence of stomates in the peritoneum of the rabbit.

In the *Arbeiten aus der Physiologischen Anstalt zu Leipzig* for 1866 appeared four papers treating either of the epithelium of the serous membranes or of their relation to the lymphatic system. They were as follows:

1. Upon the Absorption and Excretion of the Pleural Wall, by Dybkowsky.

2. Upon the Centrum Tendineum of the Diaphragm, by C. Ludwig and F. Schweigger-Seidel.

3. The Treatment of the Animal Tissues with Silver Nitrate. Upon Epithelium as well as upon the Lymph Canaliculi of v. Recklinghausen as the Supposed Origins of the Lymphatic System, by F. Schweigger-Seidel

4. Upon the Peritoneal Cavity of the Frog and its Connection with the Lymphatic System, by F. Schweigger-Seidel and J. Dogiel.

In the first three of these papers is discussed the question of the communication of the lymphatic vessels with the peri-

toneal and pleural cavities in mammals. Dybkowsky found small openings leading from the pleural cavity into the lymphatics. The others also found stomates.

The last paper mentioned above was especially helpful, both on account of the definite and careful statements made and the conclusions arrived at. The authors showed that (1) ciliated cells are present on the peritoneum of the adult female frog only; (2) the ciliated cells occur either singly or in groups among the non-ciliated cells; (3) ciliated cells are smaller and have more regular boundaries than the non-ciliated cells; (4) ciliated cells stain more deeply with silver nitrate than the non-ciliated cells; (5) the direction of the current is toward the mouth of the oviduct, and they therefore conclude that the cilia are for the purpose of aiding the ova to reach the opening of the oviduct; (6) the presence of stomates was demonstrated in the *septum cisternæ lymphaticæ magnæ*.

Afonassiew ('60) denied the presence of stomates.

Klein ('72) has written much concerning the serous membranes. In 1872, in a paper on Remak's Ciliated Vesicles and Corneus Filaments in the Female Frog, he described at length the ciliated vesicles found in the mesentery and mesogastrium of the female frog. He believed they are sinuses belonging to the lymphatic vessels and that, perhaps, they become ciliated only under pathological conditions. He mentions cilia as occurring between the stomates as well as on their borders.

In Klein's comprehensive work upon the lymphatic system he treated in Part I. ('73) of the serous membranes, and in Part II. ('75) of the lung. Klein demonstrated the presence of stomates leading from the pleural and peritoneal cavities into the lymphatics, and gave a full description of both the connective tissue and the epithelium of the serous membranes. He described the ciliated, germinating epithelium of the mesogastrium of the female frog as being present during the winter months, but he neglected to state whether this was the case in the male also and whether in

the female the cells were germinating and ciliated during the summer.

Toureux ('74) made some investigations upon the *Triton* as well as the frog. He believed that the places which others took for stomates were merely depressions in the membrane.

The next year, 1875, two papers pertaining to the peritoneum of the frog appeared. The first one by Grunau ('75), treated of the ciliated epithelium upon the peritoneum and of the oviducts of the adult female frog. He found cilia upon the peritoneum lining the ventral and dorsal walls of the body cavity and upon the liver and membranes about the mouth of the oviduct and that the frequency of ciliated cells diminished as the distance from the mouth of the oviduct increased. Grunau stated that ciliated cells are smaller, but project more than the non-ciliated.

The second paper was by Neumann and Grunau ('75). They found cilia upon the peritoneum of the adult female frog and the *Triton*, the sex of the latter not being mentioned.

Toureux and Hermann ('76) considered the places which had been described by others as stomates as merely the centers of cell formation.

Nicolsky ('80) stated emphatically that "the cilia upon the epithelium of the serous membranes of the frog exist in all individuals without distinction of sex and age." He qualifies this statement, however, by saying that the cilia do not extend over so much space in the male and young female as in the adult female, and that "in the males the cilia are very short—one must search a long time for them."

Purser ('84) also went so far as to say that ciliated epithelium is found upon the peritoneum of the male frog, as well as the female, although not in such large quantities, and all this after having made the statement that the motion of the cilia is supposed to aid the ova in their passage to the mouth of the oviduct.

Some of the standard authors, such as Huxley, Quain

and Piersol, mention the occurrence of stomates upon the peritoneum in both *Mammalia* and *Amphibia* and a number treat of ciliated epithelium upon the peritoneum of the frog, but in many cases no definite data as to age and sex are given.

Ecker ('89), in his extended work upon the Anatomy of the Frog, spoke of cilia being present upon the peritoneum, but did not state definitely whether this was the case in both sexes and all ages.

Kolossow ('93) worked upon two species of *Rana*, one of toad, two species of *Triton*, one of salamander and the *Axolotl* and found cilia only in the adult female of the *Triton*, frog and *Axolotl*.

PERITONEUM.

The peritoneum is the serous membrane lining the abdominal cavity. It is a closed sac, excepting in the female, where the opening of the oviducts break the continuity. In *Amphibia* the parietal peritoneum tightly adheres to the body wall, and the visceral completely invests all the viscera, with the exception of the kidneys, which are covered only on one side. The diagrams (Figs. 1–3) show the peritoneum as it appears in sections cut through the peritoneal cavity of *Necturus* at different levels.

The peritoneum is developed from the mesoderm. According to Minot ('92) the specialised layer of the mesoderm, called mesothelium, lines the body cavity and covers parts of the viscera. At first the cells of the mesothelium are cuboidal and cylindrical, but as development goes on they become reduced in thickness and finally reach the thin plate-like form of the epithelium. From the mesothelium is also derived a layer of mesenchyma, which finally becomes the connective tissue stratum of the peritoneum.

The peritoneum is a thin shining membrane, through which the different organs can be plainly seen. It is composed of (1) the connective tissue stratum and (2) a single layer of epithelial cells.

The first consists principally of bundles of white connective tissue. In *Necturus* these form a thick network. The fibers are arranged in wavy rows, either parallel or crossing each other. (Fig. 4.) Numerous fine elastic fibers are present. They often branch, as is shown in Fig. 4. In the mesentery of the small intestine were found bundles of smooth muscle cells. Although not very numerous some were of quite good size. (Fig. 5.) A single cell is shown in Fig. 6. The nuclei are thicker and more rounded than the typical rod-shaped nuclei of smooth muscle cells. In the peritoneum lining the dorsal wall of the body cavity of *Necturus* are numerous pigment cells which branch and anastomose. (Fig. 7.) Pigment cells occur in different parts of the peritoneum in the various *Amphibia* studied. In *Diemyctylus*, the peritoneum covering the dorsal wall of the body cavity and the testes, is very densely pigmented. In all of the *Urodela* examined there was some pigment in the peritoneum, principally in the serosa of the testis, the dorsal wall of the body cavity and the mesentery of the intestine.

The epithelium of the peritoneum is a simple squamous epithelium. The cells are united by a thin layer of cell cement. For determining the character, shape and size of the epithelial cells and their relation to each other, three principal methods of investigation were employed : (1) The examination of fresh material in blood serum or normal salt solution. (2) The application of silver to the fresh material. (3 The preparation of sections. The first method is especially recommended by Klein in his work on serous membranes. It proved very advantageous in detecting the presence of cilia, as they are visible with a high power both in motion and at rest. The method of procedure is as follows: A bit of the peritoneum is spread out on a slide in a drop of blood serum or normal salt solution and covered with a coverglass, care being taken not to rub or tear the epithelium. With a 2 mm. oil immersion objective the cilia can be seen, both on the edge of the preparation in profile and also on the surface in face view. Normal salt solution proved as good a

medium as blood serum. At first the cilia move so rapidly that they can scarcely be seen, but the speed gradually slackens until they only move a few times a second and finally stop altogether. In the fresh preparations the nuclei are visible but the cells are not demarcated.

OCCURRENCE OF CILIA.

The ripe ova are shed into the abdominal cavity and must pass cephalad to reach the mouth of the oviduct. The question of the presence of cilia upon the peritoneum of *Necturus* is a very interesting and important one, as up to the present time no one has published any statements concerning the passage of the ova from the ovary to the oviducts or the finding of ova in the oviducts. According to Marshall ('93), in the frog the ova are propelled partly by the muscular action of the body walls and partly by the motion of the cilia on the peritoneum. So far as I know, the first means has not been demonstrated, and it is hard to see how the muscular action of the body walls would tend to force the ova into the oviduct. If the sole purpose of the cilia is to propel the ova towards the mouth of the oviduct, then we should expect to find cilia in the adult female and at the period of ovulation only. It is possible that cilia may be developed in the female at maturity and then remain during the rest of the animal's existence, but their presence would not be looked for in the male and young female. Hence, it seems strange that Nicolsky and Purser described cilia upon the peritoneum of the male as well as the female, even if only in small quantities.

In both sexes of *Necturus* the peritoneum was thoroughly examined for cilia in the following parts: the hepatic ligament, the ventral wall of the body cavity, the dorsal wall of the body cavity, the membranes near the mouth of the oviduct, the mesopneumon, the mesogastrium, the serosa of the stomach, the serosa of the liver, the serosa of the small intestine, the mesentery of the small intestine, the mesentery

of the large intestine, the spheno-hepatic ligament, the gastro-hepatic ligament, the gastro-splenic ligament, the serosa of the ovary, the mesoarium, the serosa of the testis, the mesorchium, the peritoneal fold supporting the oviduct, and the peritoneal fold supporting the vas deferens.

In *Necturus maculatus* cilia were found only in the adult female. The male *Necturi* were destitute of cilia upon the peritoneum (Fig. 8) and females, in which the ova were only one-third the size of those which would be laid in the spring, showed the same condition as the males. These immature females measured 28 and 29 cm., but in one female only 28 cm. in length, cilia were as long and as widely distributed over the peritoneum as in the large specimens. Although this animal was smaller than the others the ova were nearly mature. Adult female *Necturi*, 32.5 cm. in length, were examined before ovulation. In them cilia occurred in great abundance upon certain parts of the peritoneum. Cilia were constant in all the adult female *Necturi* examined from January to April on the following parts, viz., the hepatic ligament (Fig. 9), the ventral wall of the body cavity (Fig. 10), the membrane near the mouth of the oviduct (Fig. 11) and the serosa of the liver (Fig. 12).

On these parts of the peritoneum cilia were very generally distributed. Examinations were made at intervals over the surface and ciliated cells were found on every piece examined. Not every cell was ciliated, however. On the ventral wall ciliated cells were distributed from the pelvis to the cephalic end of the abdominal cavity. About one-half the number of adult female *Necturi* showed ciliated cells on the dorsal wall of the body cavity also. Where they occurred, they were distributed on the cephalic third of the dorsal wall and fewer cells were ciliated. No cilia were found in *Necturus* upon the mesentery, serosa of the ovary nor mesogastrium, although various writers have described cilia in these parts in the frog. Waldeyer ('70) spoke of cilia being present upon the mesentery of the frog, presumably in the female, as he mentioned cilia also upon the mesoarium. Thiry

('66) found cilia upon the serosa of the frog's ovary, and Klein ('73) upon the mesogastrium, and Leydig upon the mesoarium. Ecker ('89) found cilia near the openings of the oviducts, and Grunau ('75) upon the serosa of the liver of the female frog and also near the oviducts, and Neumann ('75 upon the serosa of the liver of the female frog and in *Triton cristatus*.

In specimens of adult *Amblystoma punctatum*, taken just before ovulation, cilia were found upon the hepatic ligament (Fig. 13), the ventral wall of the body cavity, the membrane near the mouth of the oviduct, the serosa of the liver, the mesoarium and the fold of peritoneum supporting the oviduct, but none upon the dorsal wall of the body cavity. Adult female *Amblystomas*, taken soon after ovulation, as well as those taken in August, showed cilia in the same places as those taken before ovulation. Cilia were present in *Amblystoma punctatum* on the hepatic ligament and the serosa of the liver (Fig. 14) during the fall. This was determined by sections cut of a preserved specimen taken in October and killed in December. Very probably cilia occurred in other places, but it was impossible to see them in the sections. In male *Amblystoma* no cilia were found. No immature females were examined. From this it is evident that in *Amblystoma punctatum* cilia persist throughout the year.

In adult female *Diemyctylus viridescens* before ovulation cilia occur upon the hepatic ligament (Fig. 15), the ventral wall of the body cavity, the membranes near the mouth of the oviduct and the serosa of the liver. No cilia were noticed upon the dorsal wall. Neither immature females nor any females after ovulation were examined. Male *Diemyctylus* showed no cilia upon the peritoneum.

In adult female *Desmognathus* cilia were present before ovulation upon the hepatic ligament, the ventral wall of the body cavity and the membranes near the mouth of the oviduct, but not upon the dorsal wall of the body cavity. A young female with immature ova possessed no cilia upon the

peritoneum. Two larval *Desmognathi* showed no trace of cilia, although serial sections were made of the whole body. The male *Desmognathus* had no cilia upon the peritoneum.

From the foregoing observations we see that, in all four species examined, cilia were always present in the adult female and that no cilia were found in the male or immature female, so far as examined.

Arrangement of Ciliated Cells.—Ciliated cells occur either singly or in groups between the non-ciliated cells. This was well shown in the fresh preparation. (Figs. 11 and 16.) The condition mentioned by Grunau of the ciliated cells being arranged in stripes radiating from the mouth of the oviduct, has not been noticed, although I found that the ciliated cells were most numerous near the mouth of the oviduct. (Fig. 11.)

Direction of Current.—To determine the direction of the current produced by the cilia, powdered carmine was placed upon the peritoneum immediately after the death of the animal. By the aid of a simple microscope it was seen to move towards the mouth of the oviduct and was finally drawn into the opening. Mrs. Gage's method of using minute blood clots instead of carmine was tried, and proved more successful than the carmine. Immediately after death a small blood clot was placed upon the peritoneum covering the ventral wall of the body cavity of an adult female *Necturus*. It traveled 10.5 mm in ten minutes in the direction of the opening of the oviduct. Three hours after death a clot traveled only 6 mm. in ten minutes. In an adult female *Necturus*, where cilia were present on the dorsal wall, the direction of the current of the cilia on the dorsal wall also was in the direction of the mouth of the oviduct. Thiry demonstrated the direction of the current in the frog as being towards the mouth of the oviduct by using black pigment.

As the ciliary currents in *Necturus* are present only in the adult female and the direction of the current is toward the mouth of the oviduct, it seems to strengthen the theory that the cilia are to aid the ova in reaching the oviduct.

The questions now arise when do the cilia make their appearance, are they persistent throughout the year and how are they developed? At present these are matters of speculation. To find out just when the cilia appear and how long they remain it would be necessary to examine a great number of specimens of all ages. As to their development there are various theories. Kolossow offers it as a suggestion that the irritation of the peritoneum, through the increase of the ovary in size about spawning time, may cause the appearance of cilia upon the peritoneum. Klein says that the "germinal cells in the female are ciliated," and Nicolsky calls the ciliated cells young epithelial cells. Schweigger-Seidel and Dogiel consider them as arising from the ciliated cells of the oviduct, while Grunau is not sure whether this is the correct view or whether the cilia are developed from the common flat epithelium of the peritoneum. Neumann holds this latter opinion, and Kolossow speaks of certain cells which are thicker than the others and covered with minute points which finally develop into cilia. This question I have not been able to decide. The ciliated cells are usually smaller but thicker than the others, but I could find no appearance of cilia just beginning to develop. There is little difference in the length of the cilia upon various parts of the peritoneum, although near the mouth of the oviduct they are generally longer.

STOMATES.

The subject of stomates has been much discussed ever since v. Recklinghausen first described them in 1862. Since that time many investigators have noticed them, both in mammals and the lower vertebrates. According to the best authorities stomates are small openings leading from the peritoneal or pleural cavities into the lymphatic vessels. They are described by v. Recklinghausen as being in the rabbit twice the size of a red blood corpuscle. Others merely say that they never exceed the size of an epithelial cell. They are usually surrounded by a radiating form of the

epithelial cells. There may occur around the stomate a number of small granular cells, which Klein ('73) called "germinating cells." In the female frog he found that these cells possessed cilia. By Schweigger-Seidel and Dogiel[1] ('66) the stomates are described as funnel-shaped, the smaller end being on the lymphatic side of the membrane. The supposed function of the stomates is to furnish a passage for the lymph from the peritoneal and pleural cavities to the lymphatic vessels.

A great diversity of opinion exists regarding the stomates. Many writers do not believe in their existence, but the prevailing opinion seems to be in favor of them. V. Recklinghausen ('63) and Dybkowsky ('66) demonstrated by experiments the presence of stomates in mammals, and Schweigger-Seidel and Dogiel ('66) in the frog. Ordmansson ('63) figures large and small stomates. Klein ('73) describes and figures stomates in the *septum cisternæ lymphaticæ magnæ*.

No doubt there is great danger of mistaking openings or precipitates for true stomates. Piersol said that sometimes the end of one of the processes of a connective tissue cell may protrude between the epithelial cells and, taking a brown stain with silver nitrate, be easily mistaken for a stomate. Such appearances he, with other writers, calls pseudo-stomates or stigmata. Toureux and Hermann ('76) said the so-called stomates are merely depressions in the membrane, which become filled with an albuminous liquid and so form a precipitate with silver nitrate. If such is the case, by thoroughly rinsing with distilled water these appearances may be avoided.

Stomates are especially abundant in the *septum cisternæ lymphaticæ magnæ* of the frog. This I have found to be the case in the female of *Rana Virescens* (Figs. 17-18) The cells are arranged in a radiating manner about the opening, the nucleus in nearly every case being situated in the end of the cell bordering on the stomate. No small cells are present around the opening. In *Necturus maculatus* there is

no *cisterna lymphatica magna* and I could find no stomates anywhere in the peritoneum, although various methods of injection were tried and microscopical examinations of both the fresh and hardened material were made. Certain appearances (Fig. 19) I could not account for, but they are probably artificial.

FORM AND SIZE OF CELLS.

To bring out the boundaries of the cells v. Recklinghausen's silver method was adopted. Perfectly fresh material was used, as it was found that tissue which had been used for the examination in normal salt solution did not silver well, the salt and blood serum forming a precipitate with the silver nitrate, even though care was taken to rinse it well with distilled water before silvering. In membranes which had silvered in bright sunlight the lines of cell cement were sharp and dark, but when the process went on slowly in a dim light the effect was not so good as a brown precipitate was formed over much of the surface of the cells.

Ciliated cells stain more deeply than the non-ciliated (Fig. 8). Whether this is due to the presence of cilia or to albumen being retained between the cilia is at present unknown.

The cells of the epithelium vary greatly in form from the almost regular hexagonal shape of the cells of the serosa of the liver (Fig. 20) and testis (Fig. 21) of *Necturus* to the very serpentine form of the mesentery of *Diemyctylus* (Fig. 22). The number of sides varies from four to seven, the most common number being five or six. The difference in form is in a great measure due to the stretching or non-stretching of the tissue.

Schwartz, in an article in the *Anatomischer Anzeiger* for 1893, proved that this was so in the case of the peritoneal epithelium of the cat and the rabbit. He showed that when the organs were distended the epithelial cells of the peritoneum surrounding them were larger and of more regular outline than when the organs were empty. This was tried

on the mesentery of *Necturus* and *Diemyctylus* by stretching and hardening the membrane and then comparing the form of the cells with those from part of the same membrane which had not been stretched. In the first case (Fig. 23) the cells were larger and quite regular in outline, while in the mesentery, which had not been stretched (Fig. 24), the cells were very sinuous in form and so contracted that they appeared smaller than the others.

There was no appreciable difference in the form or size of the epithelial cells between the male and female. In every case noticed in *Necturus* the cells on the dorsal wall of the body cavity (Fig. 25) were larger than those on the ventral wall (Fig. 10). Ciliated cells are usually smaller than the non-ciliated cells, but thicker, so that they project more than the others (Figs. 9 and 10). In *Necturus* the cells of the serosa of the liver and other solid organs are more regularly hexagonal than on any other part. Probably this is due to the fact that these organs are more uniform in shape and size than the others. The shape of the cells of the hepatic ligament, serosa of the liver, mesopneumon and membrane between the spleen and lungs and between the liver and lungs are shown in Figs. 26, 27, 28, 29 and 30.

Measurements.—In length the cells averaged 57.3 μ., in breadth, 39.8 μ., while the average thickness was 12.31 μ. The average size of the nuclei near the mouth of the oviduct in *Necturus* was, length, 38 7 μ., width, 22.5 μ., and thickness, 8.75 μ. The average length of the cilia upon the hepatic ligament in the female *Necturus* was 3 12 μ. and of those upon the membranes near the mouth of the oviduct was 3.75 μ.

NUCLEI.

The nuclei are usually large, oval or kidney-shaped. They are nearly always placed eccentrically and generally the nuclei of two adjacent cells are in juxtaposition. The cells project opposite the nuclei. In the epithelium of the peri-

toneum, covering the contracted stomach and intestine of *Necturus*, a peculiar condition was noticed. The rows of cells seemed to be arranged in pairs. In each pair the nuclei were juxtaposited so that there was the appearance of the nuclei being situated in double rows (Fig. 31). But when the organ was distended this appearance was lost (Fig. 32). Occasionally a cell with two nuclei was noticed (Fig. 33).

SUMMARY.

The result of this investigation may be summed up as follows :

1. The peritoneum is made up of two parts, viz., the connective tissue stroma and the epithelium.

2. The connective tissue stroma is composed of white connective tissue, elastic fibers and bundles of smooth muscle cells.

3. The peritoneal epithelium was ciliated only in the adult females studied. It was impossible to demonstrate cilia in the males or young females, although their presence upon the peritoneum of some *Amphibia* has been described by a few writers.

4. All the specimens of *Necturus*, *Desmognathus* and *Diemyctylus* were taken from January to April and none were examined after spawning. Specimens of *Amblystoma* were examined both before and shortly after spawning and in August, and one specimen which was taken in October and prepared in December.

5. In the adult females of all the species named taken in the spring and in August, cilia were constant upon the following parts : Hepatic ligament, the ventral wall of the body cavity, the membranes near the mouth of the oviduct and the serosa of the liver.

6. In *Necturus* some of the adult females showed cilia also upon the dorsal wall of the body cavity.

7. Specimens of adult female *Amblystomas* taken in the spring and in August differ from the other species in having cilia upon the mesoarium and the membrane supporting the oviduct.

8. In a single example of adult female *Amblystoma* taken in October and prepared in December, cilia were present upon the hepatic ligament and the serosa of the liver.

9. The direction of the current produced by the cilia is toward the mouth of the oviduct.

10. As indicated by the current the function of the cilia is probably to carry the ova to the oviducts.

11. Blood clots were carried by the cilia on the ventral wall immediately after death at the rate of 10.5 mm. in ten minutes, and three hours after death at the rate of 6 mm. in ten minutes.

12. No stomates were found in *Necturus maculatus.*

13. The shape of the cells varies greatly, but most of this variability is due to the stretching or non-stretching of the tissue.

14. The size of the cells varies in different parts of the peritoneum and in different species.

15. The cells on the dorsal wall of the body cavity are usually larger than those on the ventral wall.

METHODS.

The specimens were killed in 1-6 chromic acid, ether being added to the proportion of 5 c.c. to the liter. The abdomen was then opened and the various parts of the peritoneum were examined for the presence of cilia. The tissues were kept moist with blood serum or normal salt solution.

Bits of clotted blood or finely powdered carmine were used to determine the direction of the ciliary currents.

Other specimens which had not been utilised in the above

manner were treated with silver nitrate. After the abdomen was opened and the specimen thoroughly washed in distilled water to remove the blood and lymph, a 1-5 per cent. solution of silver nitrate was poured over it and the viscera carefully moved so as to allow the silver to reach every part of the peritoneum. This was permitted to remain two or three minutes, or until the peritoneum had a silvery cast. Then after several washings with distilled water the specimen was placed in a porcelain dish with plenty of distilled water in the direct sunlight or the strongest daylight until the peritoneum was of a light brown color. In the bright sunlight only a few minutes were required.

For a nuclear stain Gage's hematoxylin (Gage, 1892,) was the most successful. In preparations showing the face view of the epithelium no stain beside hematoxylin was used. In the sections eosin was used as a counter stain. For the study of the connective tissue, the membrane was gently penciled to remove the epithelium. A 2 per cent. acetic acid caused the white connective tissue to swell up and become transparent, so the elastic fibers which were unaffected by the acid stood out plainly. Van Gieson's picric acid fuchsin (Freeborn, 1893,) was used to stain the white connective tissue.

The tissue was hardened simply in alcohol, 50, 67 and 82 per cent. being used, the time for the first two being one to three days and the tissues were left in 82 per cent. until needed. Picric alcohol 1 to 3 days and then 67 and 82 per cent. alcohol was also used, but the picric acid tended to obscure the cilia.

Hardened tissues were mounted in balsam and glycerine jelly was used for the fresh.

Both the paraffin and collodion methods were used in imbedding and sectioning.

The larval forms were hardened, some in Fish's picro-aceto-sublimate, and others in formalin and imbedded whole in collodion and sectioned.

For the dissociation of the epithelial cells various reagents

were tried, but equal parts of Gage's picric alcohol and water, 2 to 4 hours, proved the most successful.

To determine whether the change in the form of the cells was due to the stretching of the membrane the following method was employed. Pieces of the fresh mesentery and hepatic ligament of *Necturus* were stretched over Hoggan's histological rings, treated with silver in the usual way and hardened. The mesentery of *Diemyctylus* being too small to cover a ring, the necks of small homeopathic vials were used for this instead of rings. The small intestine of *Necturus* was stretched by distending it and then hardening.

BIBLIOGRAPHY.

This list includes only such books and papers as were helpful in this investigation. Extended bibliographies are given by Klein ('73 and '75), Hoffman ('78), and Ecker ('89).

'68. Afonassiew, N.—Ueber den Anfang der Lymphgefässe in den serösen Häuten. Virchow's Arch., Bd. XLIV. (1868), pp. 37–68, Taf. III. and IV.

'75. Arnold, Julius—Ueber das Verhalten der Wandungen der Blutgefässe bei der Emigration weisser Blutkörper. Virchow's Arch., Bd. LXII. (1875), pp. 157–194, Taf. III.

'76. Arnold, Julius—Ueber die Kittsubstanz der Endothelien. Virchow's Arch., Bd. LXVI (1876), pp 77–109, Taf. V

'78. Arnold, Julius—Ueber die Durchtrittsstellen der Wanderzellen durch entzündeteseröse Häute. Virchow's Arch., Bd. LXXIV., pp. 245–267, Taf. VII.

'85. Baker, Frank—Peritoneum. Reference Handbook of the Medical Sciences. A. H. Buck, Editor. Vol. V. (1885), pp. 600–606.

'67. Cohnheim, J—Ueber Entzündung und Eiterung. Virchow's Arch., Bd. XL. (1867), pp. 1–79.

'89. Cope, E. D.—The Batrachia of North America. Bull. No. 34, U. S. National Museum. Washington, D. C. (1889).

'66. Dybkowsky—Ueber Aufsaugung und Absonderung der Pleurawand. Arb. aus der Phys. Anstalt zu Leipzig. Bd. I. (1866), pp 40–67. 1 plate.

'89. Ecker, Alex.—The Anatomy of the Frog. Translated with additions by George Haslam. London (1889).

'75. Foa, P.—Ueber die Beziehung der Blut und Lymphgefässe zum Saftkanalsystem. Virchow's Arch., Bd. LXV. (1875), pp. 284–300.

'91. Gage, S. H.—Life History of the Vermilion spotted Newt (*Diemyctylus viridescens*. Raf). Amer. Nat., Dec, 1891, pp. 1084–1110.

'92. Gage, S. H.—An Aqueous Solution of Hematoxylin which does not Readily Deteriorate. Proc. Amer. Soc. of Micr., Vol XIV (1892), pp. 121–127.

'95. Gage, S. H.—Improvements in Oil-sectioning with Collodion. Proc. Amer. Micr. Soc, Vol. XVII (1895), pp. 361-370.

'94. Gould, Geo. M.—An Illustrated Dictionary of Medicine, Biology and Allied Sciences. Philadelphia (1894).

'75. Grunau, Hermann—Ueber das Flimmerepithel auf dem Bauchfell des weiblichen Frosches und ueber den Eileiterbau desselben. Dissert. Königsberg (1875).

'78. Hoffman, C. K.—Bronn's Klassen und Ordnungen des Thierreiches. Vol. VI. (1878), Part II., Amphibia.

'75. Huxley, T. H—Article: Amphibia, in the Encyclopedia Brittanica. Vol. I. (1875).

'72. Klein, E. B.—On Remak's Ciliated Vesicles and Corneous Filaments of the Peritoneum of the Frog. Quar. Jour. Mic. Soc., Vol. XII. (1872), pp 43-50.

'73. Klein, E.—Handbook for the Physiological Laboratory. Edited by J Burdon-Sanderson Vol I., Text. Vol. II., Plates (1873).

'73-'75. Klein, E. B.—The Anatomy of the Lymphatic System. Part I., The Serous Membranes. London (1873). Part II., The Lung London (1875).

'80. Klein, E, and Smith, E. N—Atlas of Histology. London (1880).

'93. Kolossow, A.—Ueber die Struktur des Pleuroperitoneal und Gefässepithels. (Endothels.) Arch. Mikr. Anat., Bd. XLII. (1893), pp. 318-383, Plate XXII.

'93. Lee, A. B.—The Microtomist's Vade Mecum. Third Edition (1893.)

'57. Leydig.—Lehrbuch der Histologie der Menschen und der Thiere. (1857.)

'66. Ludwig, C., and F. Schweigger-Seidel—Ueber das Centrum Tendineum des Zwerchfells. Arbeit aus der Phys. Anst. zu Leipzig, Vol. I. (1866), pp. 174 181. 1 plate.

'93. Marshall, A. Milnes—Vertebrate Embryology, p. 99. New York. (1893.)

'32-'36. Mayer, ———.—Ueber die Flimmerbewegung. Frorieps Notizen, Nr. 1024, Seite 179, and Nr. 1028, Seite 247.

'93. Metchnikoff, Elias—Lectures on Comparative Pathology of Inflammation, delivered at the Pasteur Institute in 1891.

'57. Milne-Edwards, A.—Leçons sur la Physiologie et l'Anatomie Comparèe de l'Homme et des Animaux. T. IV., pp 462. T. V., pp. 216 and 268. T. VI., pp. 4, 5, and 368-378. (1857.)

'92. Minot, Chas. Sedgwick—Human Embryology. New York. (1892).

'94. Muscatello, G.—La signification physiologique de la forme des Endotheliums. Anat. Anz, Bd. X. (1894-5), pp. 173-176. 2 Figs.

'75. Neumann, E. and H. Grunau—Die Beziehungen des Flimmerepithels der Bauchhöhle zum Eileiterepithel beim Frosche. Arch. Mikr. Anat., Bd. XI. (1875), pp. 354-378. 1 plate.

'80. Nicolsky, P.—Ueber das Flimmerendothel beim Frosche. Centrbl. f. d. Med. Wiss., August, 1880, p. 641.

'63. Ordmansson, Ernst—Beitrag zur Lehre von dem Epithel. Virchow's Arch., Bd XXVIII. (1863), pp. 361-369, Plate VII.

'85. Prudden, T. Mitchell—Endothelium. Reference Handbook of the Medical Sciences. A. H. Buck, Editor. Vol. II. (1885), pp. 689-691. New York.

'85. Prudden, T. Mitchell—Epithelium. Reference Handbook of the Medical Sciences. A. H. Buck, Editor. Vol. II. (1885), pp. 709-713. New York.

'84. Purser, J. M.—A Manual of Histology and of Histological Methods. London (1884).

'90-'91. Quain's Elements of Anatomy. Vol I., Part I., Embryology, by Prof. Schäfer. London and New York (1890). Vol. I., Part II., General Anatomy and Histology, by Prof. Schäfer, London and New York (1891).

'75. Ranvier, L.—Traite Technique d'Histologie, pp. 225-270 and 641-668. Paris, (1875).

'41. Remak, ——— .—Ueber Wimperblasen und Hornfäden. Arch. f. Anat. u. Phys. (1841), pp 441-453.

'62. Recklinghausen, F. von—Die Lymphgefässe und ihre Beziehung zum Bindegewebe. S. 22. Berlin (1862).

'63. Recklinghausen, F. von—Zur Feltresorption. Virchow's Arch., Bd. XXVI. (1863), pp. 172-208.

'77. Schäfer, Edward Albert—A Course of Practical Histology, pp. 172-184, (1877).

'91. Schiefferdecker, P., and Kossel, A.—Gewebelehre mit besonderer Berücksichtigung des Menschlichen Körpers. Erste Abtheilung mit 214 Text Abbildungen (1891).

'93. Schwartz, W. Grössen und Formveränderungen einiger Endothelien durch Dehnung. Anat. Anz., Bd. VIII. (1893), pp. 71-75.

'66. Schweigger-Seidel, F.—Die Behandlung der thierischen Gewebe mit Argent. nitric. Ueber Epithelien ueber die v. Recklinghausenschen Saftkanälchen, als die vermeintlichen Wurzeln der Lymphgefässe. Arbeit aus der Phys. Anst. zu Leipzig. Vol. I. (1866), pp. 150-173. 1 plate.

'66. Schweigger-Seidel, F. Dogiel, J.—Ueber die Peritonealhöhle bei Froschen und ihren Ausammenhang mit dem Lymphgefässsysteme. Arb. aus der Phys. Anst. zu Leipzig. Vol. I. (1866 , pp. 68-76. 1 plate.

'35. Sharpey, W.—Cilia. Todd & Bowman's Encyclopedia, Vol I. (1835,) pp. 607-638.

'62. Thiry, ——— .—Ueber das Vorkommen eines Flimmerepitheliums auf dem Bauchfell des weiblichen Frosches. Goettinger Nachrichten (1862).

'74. Tourneux, F.—Recherches sur l'epitheliums des sereuses. Jour. de l'Anat. et Phys., Vol. X. (1874), pp 66-83, Plates I. and II. Paris.

'76. Tourneux and Hermann—Recherches sur quelques epitheliums plats dans la serie animale. Jour. de l'Anat. et Phys., Vol. XII. (1876), pp. 199-221, Plates V. and VI. Paris.

'42. Valentin, Gabriel G.—Flimmerbewegung. Extract from Der Handwörterbuch der Physiologie, Bd. I. Braunschweig (1842) (?).

'70. Waldeyer—Eierstock und Ei. Leipzig, (1870).

'83. Waldeyer—Archiblast und Parablast. Arch. f. Mikr. Anat., Bd. XXII., (1883), pp. 1-77.

'75. Wiedersheim, A.—*Salamandrina perspicillata* und *Geotriton fuscus*. Genua (1875).

'86. Wiedersheim, R —Elements of the Comparative Anatomy of Vertebrates. Adopted from the German by W. Newton Parker. London and New York (1886).

'93. Freeborn, G. C.—Van Gieson's Picro-Acid Fuchsin as a selective stain for Connective Tissue. N. Y. Path. Soc., 1893, p. 93, Vol. IV. Studies from the Dept. of Pathology of the College of Physicians and Surgeons, Columbia College, N. Y., Vol. IV , for the Collegiate year 1894-1895.

PLATE I.

Fig. 1. Transection through the cephalic part of the abdominal cavity of *Necturus maculatus*, showing the peritoneum and its relation to the body wall, liver, stomach, lungs and oviduct.

a. = white connective tissue fibers.	*ov.* = ovary.
b. = elastic fibers.	*p.* = peritoneum.
c. = pigment cells.	*st.* = stomach.
o. = oviduct.	*v.* = postcava.
s. = spleen.	*x.* = dorsal aorta.
i. = small intestine.	*y.* = portal vein.
e. = lung.	*l.* = liver.

Fig. 2. Transection through the body cavity of *Necturus* farther cephalad than Fig. 1.

Fig. 3. Transection through the body cavity of *Necturus* at middle of small intestine.

Fig. 4. Fresh mesentery of small intestine of *Necturus maculatus*. Epithelium removed by penciling.

Fig. 5. Bundle of smooth muscle cells from mesentery of small intestine of *Necturus maculatus*.

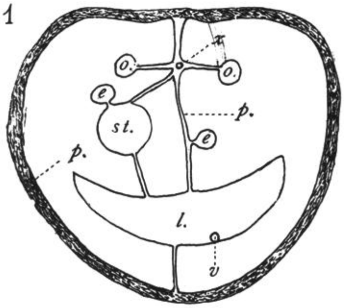
Fig. 6. Isolated smooth muscle cells from mesentery of small intestine of *Necturus maculatus*.

Fig. 7. Peritoneum from dorsal wall of body cavity of *Necturus maculatus*. Fresh, epithelium removed by penciling.

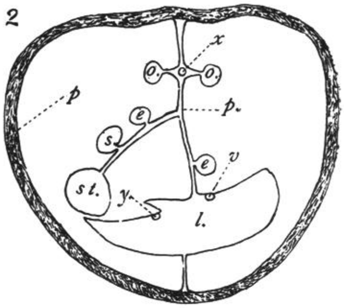
a. = ciliated cell.
b. = non-ciliated cell.
c. = cell folded over, showing cilia in profile.

PLATE I.

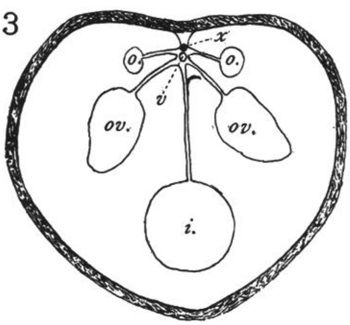
1



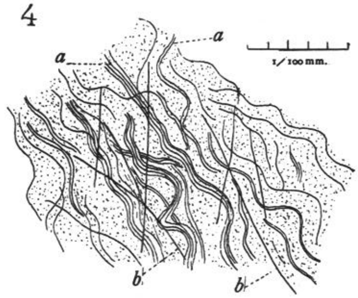
2



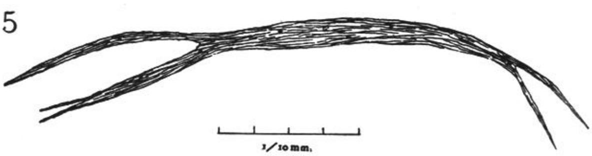
3



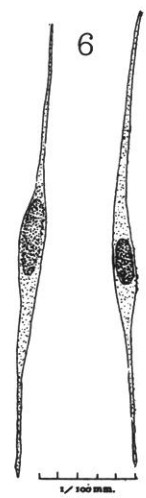
4



5



6



7

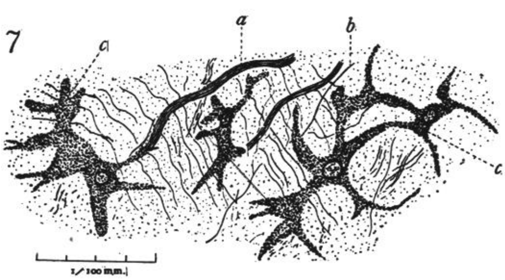


PLATE II.

Fig. 8. Cross section of the serosa of the liver of male *Necturus*, showing absence of cilia.

Fig. 9. Cross-section of the epithelium from the hepatic ligament of adult female *Necturus*, showing cilia.

Fig. 10. Surface view of peritoneal epithelium from the ventral wall of the body cavity of adult female *Necturus*.

Fig. 11. Surface view of fresh peritoneal epithelium near mouth of oviduct of female *Necturus*.

Fig. 12. Cross-section of epithelium from liver of female *Necturus*

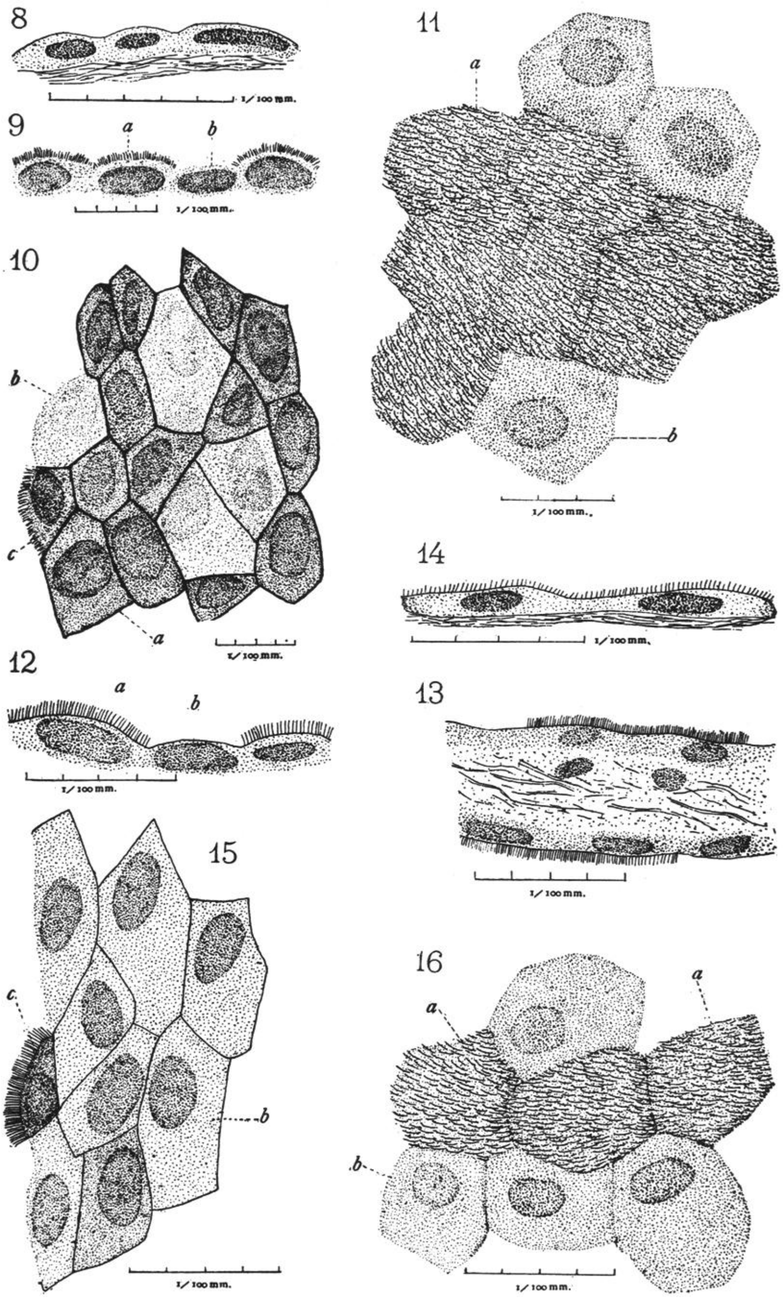
Fig. 13. Cross-section of hepatic ligament of adult female *Amblystoma*, taken just before spawning.

Fig. 14. Cross-section of serosa of liver of adult female *Amblystoma*, taken in October.

Fig. 15. Surface view of epithelium from hepatic ligament of female *Diemyctylus*.

Fig. 16. Surface view of fresh peritoneal epithelium from hepatic ligament of female *Necturus*.

PLATE II.



8
1/100 mm.
9
a
b
1/100 mm.
10
b
c
a
1/100 mm.
11
a
b
1/100 mm.
12
a
b
1/100 mm.
13
1/100 mm.
14
1/100 mm.
15
c
b
1/100 mm.
16
a
a
b
1/100 mm.

PLATE III.

Fig. 17. Peritoneal side of *septum cisternæ lymphaticæ magnæ* of *Rana virescens*.

a. — stomate.

Fig. 18. Lymphatic side of *septum cisternæ lymphaticæ magnæ* of *Rana virescens*.

a. — lymphatic opening of stomate, shown in Fig. 17.

Fig. 19. Peritoneum from dorsal wall of body cavity of male *Necturus*.

a. — appearance not accounted for.

b. — opening between epithelial cells, probably artificial.

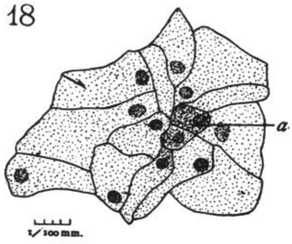
Fig. 20. Face view of epithelium from ventral surface of liver of male *Necturus*.

Fig. 21. Face view of epithelium from testis of male *Necturus*.

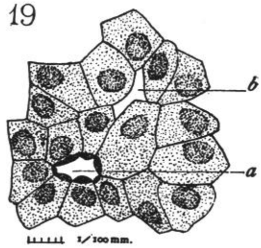
Fig. 22. Face view of epithelium from mesentery of small intestine of female *Diemyctylus*.

PLATE III.

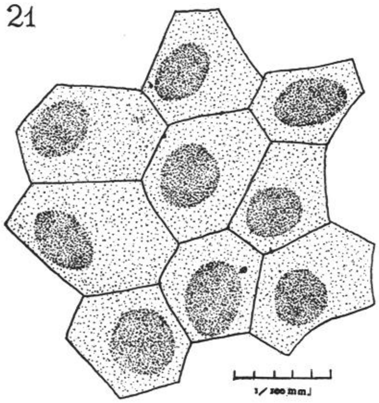
18



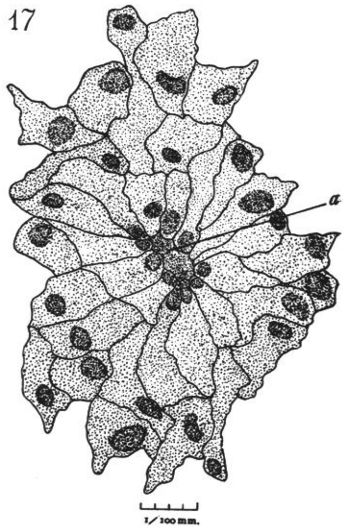
19



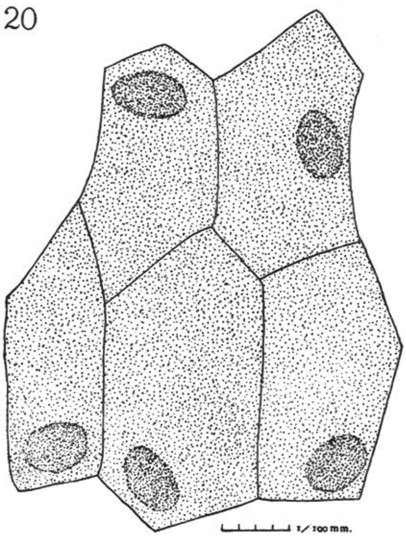
21



17



20



22

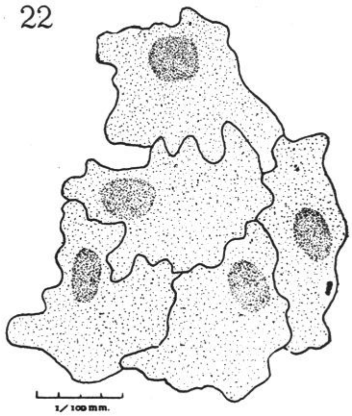


PLATE IV.

Fig. 23. Face view of epithelium from the stretched mesentery of small intestine of male *Diemyctylus*.

Fig. 24. Face view of epithelium from the unstretched mesentery of small intestine of male *Diemyctylus*.

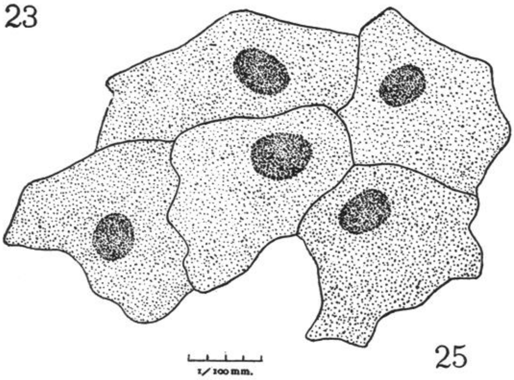
Fig. 25. Face view of epithelium from the dorsal wall of the body cavity of male *Necturus*.

Fig. 26. Face view of epithelium from the hepatic ligament of female *Necturus*.

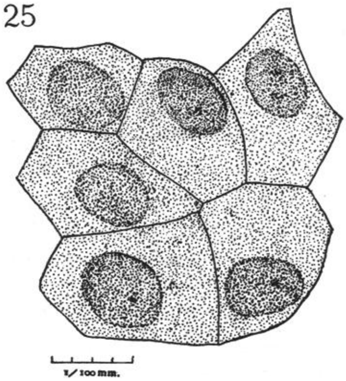
Fig. 27. Face view of the epithelium from the mesopneumon of male *Necturus*.

PLATE IV.

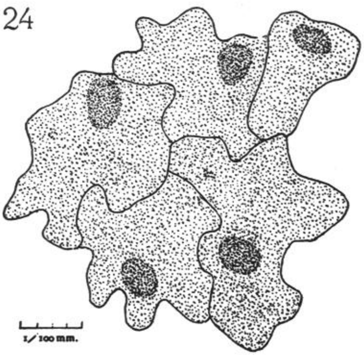
23



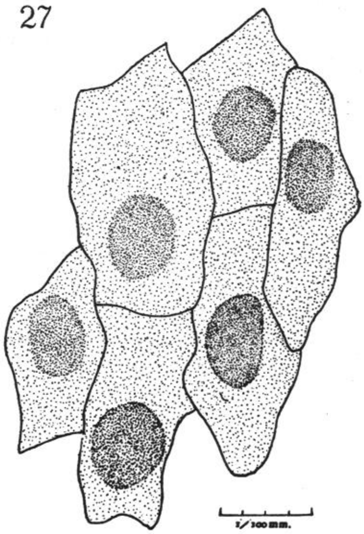
25



24



27



26

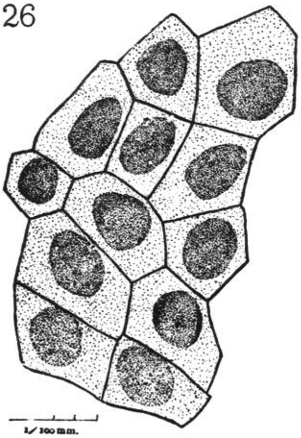


PLATE V.

Fig. 28. Face view of epithelium from the membrane between spleen and lungs of male *Necturus*.

Fig. 29. Face view of epithelium from the liver of male *Desmognathus*.

Fig. 30. Face view of epithelium from the mesopneumon of female *Diemyctylus*.

Fig. 31. Face view of peritoneal epithelium from the contracted small intestine of male *Necturus*.

a. — peculiar arrangement of the nuclei.

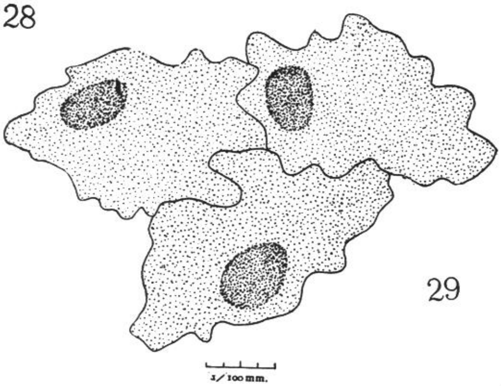
Fig. 32. Face view of the peritoneal epithelium from the distended small intestine of the male *Necturus*.

Fig. 33. Epithelial cell from the hepatic ligament of the female *Necturus* with two nuclei.

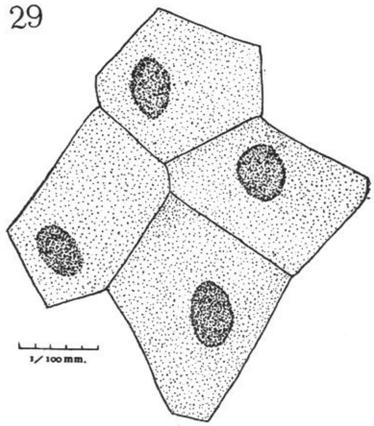
Fig. 34. Isolated epithelial cells from the hepatic ligament of male *Necturus*.

PLATE V.

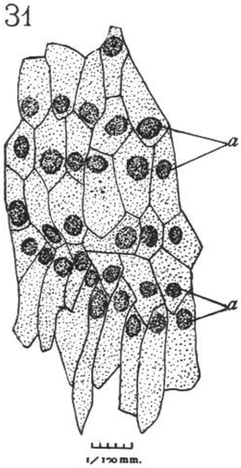
28



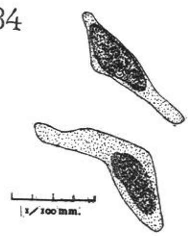
29



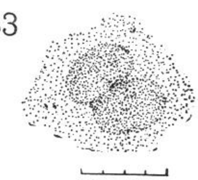
31



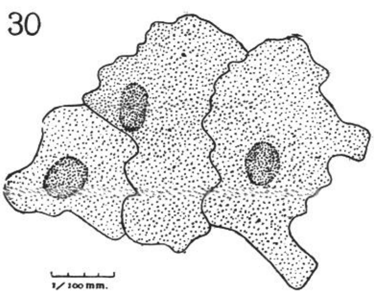
34



33



30



32